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Morphological Characteristics of the Dorsal Skin of Two Hynobiids and Their Adaptive Role in Aquatic and Terrestrial Habitats

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Abstract The morphological characteristics of the dorsal skin of trunk in two species of hynobiid salamanders, *Batrachuperus pinchonii* and *Hynobius chinensis* were examined by light microscopy. The basic structures of the skin in the two species are similar and consist of two layers: epidermis and dermis. The epidermis consists of stratum corneum, stratum intermedium and stratum germinativum, while the dermis is composed of a stratum spongiosum and stratum compactum. However, some species-specific variation has been identified (e.g., the distribution of capillary vessels and gland cells, and the thickness of skin). *H. chinensis* is a terrestrial species and only lives in water during breeding period, but *B. pinchonii* is aquatic and remains aquatic throughout its lifetime. The differences in the distribution of capillary vessels and gland cells are related to their different habitats, and show a morphological adaptation.

Keywords hynobiid salamander, skin, morphology, adaptation, habitat

1. Introduction

The skin of amphibians is the interface between the animal and its environment. Since amphibians occur throughout a wide range of different environments, the skin must be an adaptable organ system that performs several functions, such as mechanical protection (Farquhar and Palade, 1965; Bueno *et al.*, 1981; Fox, 1986a, b; Delfino *et al.*, 1995; Almeida *et al.*, 2007), respiration (Duellman and Trueb, 1994), ionic transport (Stiffler *et al.*, 1997; Sullivan *et al.*, 2000; Hillyard *et al.*, 2007), and water transport (Hoffman and Katz, 1999; Sullivan *et al.*, 2000; Azevedo *et al.*, 2007; Hillyard *et al.*, 2007). These functions are reflected in the morphological complexity of the skin as well as in the physiological properties of the underlying integumentary structures (Greven *et al.*, 1995; Bingol-Ozakpinar and Murathanoglu, 2011).

The family Hynobiidae, with 58 species belonging to nine genera, is one of the most diverse families in Urodela (AmphibiaWeb, 2013). Hynobiids are considered to be very basal salamanders within Urodela (Gao and Shubin, 2001; Wiens *et al.*, 2005; Frost *et al.*, 2006; Roelants *et al.*, 2007; Wiens, 2007, 2011; Pyron and Wiens, 2011) and can be divided into two types according to their living habits, aquatic (including all the species of the genera *Onychodactylus*, *Ranodon*, *Paradactylodon*, *Batrachuperus*, *Liua* and *Pachyhynobius*, which live in water for the whole lifetime or most of the lifetime), and terrestrial (including the species of *Salamandrella*, *Pseudohynobius* and *Hynobius*, which live in water only in breeding period). Presently, little information about skin morphology in hynobiid salamanders is available. Only *Ranodon sibiricus* (Du, 1992; Wu *et al.*, 1995), *Pachyhynobius shangchengegnsis* (Wu and Lv, 1994), "*Onychodactylus fischeri*" (Lu *et al.*, 2004; Guo *et al.*, 2009; now nominated as *O. zhaoermii* by Poyarkov *et al.*, 2012), *Hynobius retardatus* (Ohmura and Wakahara, 1998), and *Batrachuperus pinchonii* (Li and Fang, 1992) have been studied in this aspect over the last two decades.

The structure of the skin reflects its functional role in

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the microhabitat of the species under study (Spermann, 1973; de Brito-Gitirana and Azevedo, 2005; Wells, 2007). In this paper, to understand the specific morphological characteristics of the skin of hynobiid species and the relationship between the animals and their surroundings, morphological characteristics of the dorsal skin of *B. pinchonii* and *Hynobius chinensis*, belonging to aquatic type and terrestrial type, respectively, were compared and studied by light microscopy.

2. Materials and Methods

Adult male *B. pinchonii* were collected from Qiaoqi, Baoxing County, Sichuan, China by Jianli XIONG on 9 April 2006. Adult male *H. chinensis* were collected from Gaojiayan, Changyang County, Hubei by Qin CHEN. The animals were preserved in 10% formalin or 70% alcohol. The vouchers are deposited at the Henan University of Science and Technology Museum (HNUSTM) and Chengdu Institute of Biology, Chinese Academy of Sciences (CIB).

Skin samples for the two species were removed from the dorsal regions of the trunk, and fixed in 10% buffered formalin overnight at room temperature, dehydrated in a graded series of ethanol, embedded in paraplast, and sectioned at 5 μm thickness with Leica RM 2135 type microtome (Leica Microsystems, Wetzlar, Germany). Sections were stained with Haematoxylin-Eosin (HE) and observed under an OLYMPUS YM200 light microscope (LM). Parameters of the skin were measured by micrometer and MiPrd microscopic image analysis software. The data were expressed as mean ± standard deviations (SD). To compare the differences in skin morphological characteristic parameters between the two species, a Student's t test was used. The differences were significant at $P < 0.05$.

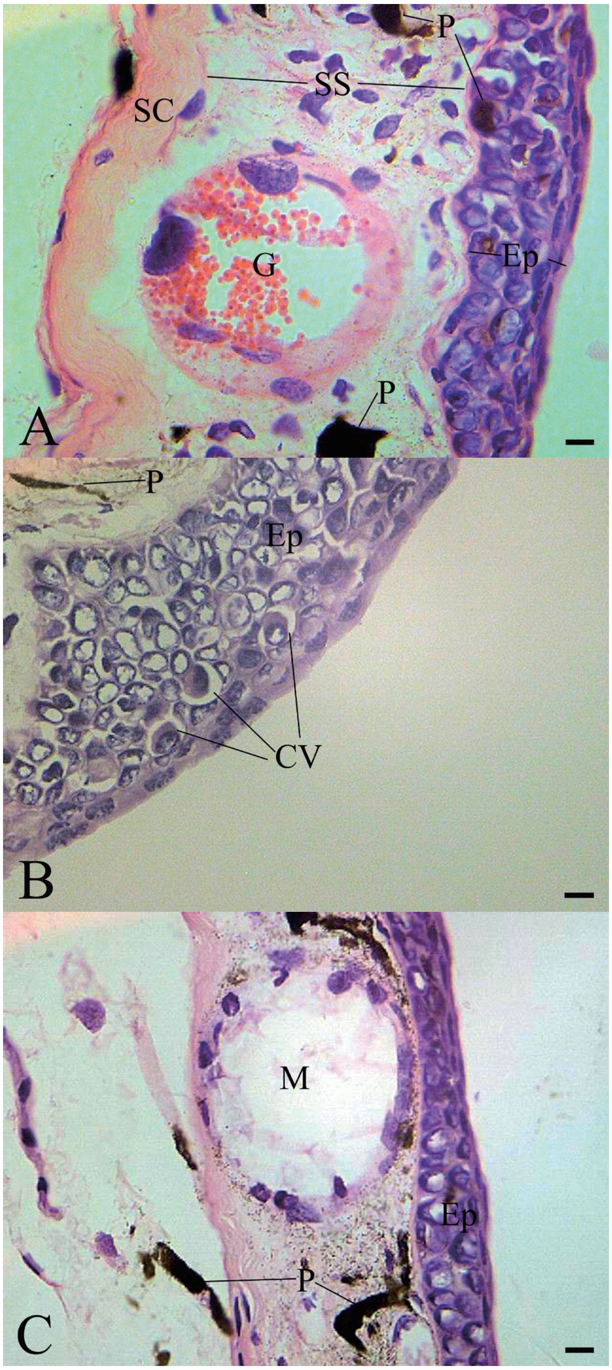

Figure 1 Light micrographs of the dorsal skin of *Batrachuperus pinchonii*. A: Shows the epidermis, pigment cells and mucous glands; B: Indicates the epidermis, pigment cells and capillary vessels; C: Shows the epidermis, pigment cells, stratum compactum, stratum spongiosum and granular glands. Epidermis (Ep); stratum spongiosum (SS); stratum compactum (SC); mucous glands (M); granular glands (G); pigment cells (P); and capillary vessels (CV). Scale bar = 20 μm. The same below.

3. Results

The basic structures of the skin of the two hynobiid species are similar (Figures 1, 2), consisting of two firmly attached layers: the outer epidermis and the inner dermis. The total thickness of the skin is significantly different ($P < 0.001$) between the two species (Table 1).

The epidermis consists of stratified epithelial cells in 3–8 layers in *B. pinchonii* and in 2–6 layers in *H. chinensis*. Due to the difference in the number of the layers, the thickness of the epidermis between the two species is significantly different ($P < 0.001$) (Table 1). Though the thickness of the epidermis differs, the epidermis can be divided into stratum corneum, stratum intermedium (or transition layer) and stratum germinativum. The stratum corneum, the outermost layer of the epidermis, consists of a single layer of flattened and

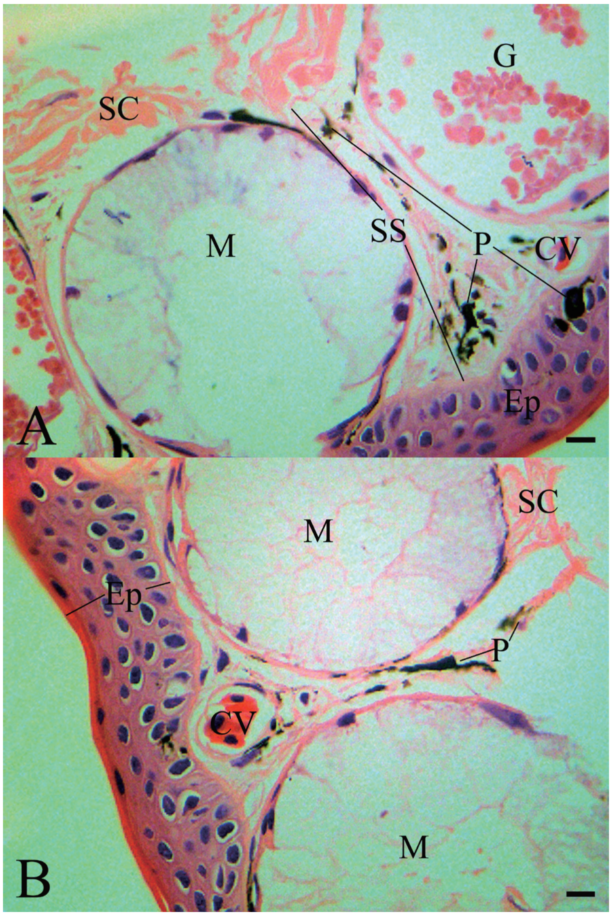

Figure 2 Light micrographs of the dorsal skin of *Hynobius chinensis*. A: Shows the epidermis, pigment cells and mucous glands; B: Shows the epidermis, pigment cells and capillary vessels.

keratinized cells, which are more acidophilic due to their keratin content and lack of nuclei. Underlying the stratum corneum is the stratum intermedium, which is composed of various layers of polyhedral cells. The stratum germinativum, basal to the others, is made of a single layer of cuboidal or cylindrical cells. Both the pigment cells and capillary vessels are discovered in the epidermis of *B. pinchonii*, but only pigment cells are found in the epidermis of *H. chinensis*.

The dermis consists of two layers, the stratum spongiosum (or spongious dermis) and the stratum compactum (or compact dermis). The stratum spongiosum, the outer layer, is composed of a loose network of collagen and elastic fibers, and contains blood vessels, pigment cells and glands. The gland cells include mucous glands and granular glands, both of which are multicellular glands, and are discovered in the stratum spongiosum of *B. pinchonii* and *H. chinensis*. The glandular secretory portion of both exocrine glands was surrounded by myoepithelial cells. The size of the gland cells differed between the two species (Table 2). Below the stratum spongiosum is the stratum compactum, which is formed by a series of alternating layers of collagenous fiber bundles arranged in a crisscrossed manner, including capillary vessels and pigment cells. The stratum spongiosum and stratum compactum are varied in thickness (Table 1), and both are significantly different ($P < 0.001$) between the two species.

4. Discussion

Although amphibian skin is the structural and functional interface between the animals and their surroundings, its morphological and functional complexity is still incompletely understood (Duellman and Trueb, 1994). In this study, the basic morphological structures of the dorsal skin of adult *B. pinchonii* and *H. chinensis* are similar, and also similar to those of other hynobiid species (Du, 1992; Li and Fang, 1992; Wu and Lv, 1994; Wu *et al.*, 1995; Ohmura and Wakahara, 1998; Lu *et al.*, 2004; Guo *et al.*, 2009). However, some species-specific differences have been identified (e.g., the distribution of capillary vessels and gland cells, and the thickness of skin). The differences in the distribution of capillary vessels and gland cells may be related to long-term adaptation and evolution under different eco-environments (Fei *et al.*, 2006; Wells, 2007).

For amphibians, the skin is an important organ for respiration (Trueb, 2003), with most gas exchange occuring through the skin (Duellman and Trueb, 1994; Trueb, 2003). The distribution of capillary vessels in the skin allows for the efficient diffusion of oxygen from the environment to the blood (Noble, 1931; Duellman and Trueb, 1994). The capillary vessels of Cryptobranchidae

Table 1 The morphometric analysis of the dorsal skin of *B. pinchonii* and *H. chinensis* (μm, Mean ± SD; Range).

Species	Total thickness	Epidermis	Stratum spongiosum	Stratum compactum
B. pinchonii	193.60 ± 64.19	85.31 ± 62.26	90.86 ± 15.35	17.42 ± 13.48
	(125.01 – 343.96)	(26.08 – 227.32)	(66.37 – 132.32)	(6.18 – 66.30)
H. chinensis	286.19 ± 51.17	49.23 ± 9.65	195.78 ± 45.10	41.18 ± 17.45
	(159.808 – 353.14)	(31.898 – 74.08)	(105.378 – 295.15)	(18.018 – 98.17)
P - value	< 0.001	< 0.001	< 0.001	< 0.001

Table 2 The sizes of the granular and mucous glands (mean) in *B. pinchonii* and *H. chinensis* (unit: µm).

Species	Granular glands	Mucous glands
B. pinchonii	170.81 × 121.34	143.22 × 104.62
H. chinensis	234.99 × 171.41	148.97 × 131.23

penetrate almost to the surface of the thick epidermis (Noble, 1931). The capillary vessels are present in the epidermis and dermis of *B. pinchonii* (Figure 1), in the epidermis and dermis of *P. shangchengensis* (Wu and Lv, 1994) and *O. zhaoermii* (Lu *et al.*, 2004; Guo *et al.*, 2009), but only occur in the dermis of *H. chinensis* (Figure 2), which may be correlated to its habitat. *H. chinensis* is a terrestrial species and only lives in water during breeding period, but *B. pinchonii* is aquatic and remains aquatic throughout its lifetime. The oxygen content of the atmosphere is approximately 21%, so oxygen is seldom a limiting factor for terrestrial species in the terrestrial environment. However, the oxygen content in fully saturated water is only about 1/30 of that in atmosphere, so aquatic habitat maybe less favorable for oxygen uptake than terrestrial one. The capillary vessels present in the epidermis of *B. pinchonii* can help assist breathing. This agrees with the result that highly aquatic salamanders probably take up most of their oxygen through the skin (Wells, 2007).

Secretions from skin glands (derived from the ectoderm) of amphibians are an important substance for courtship, sex recognition, other aspects of chemical communication, and protection, especially in urodeles (Houck and Sever, 1994; Erspamer, 1994; Clarkke, 1997). All amphibians have two major types of glands in the dermis: granular glands and mucous glands. The granular glands secrete various proteinaceous substances that serve primarily as defensive chemicals to protect amphibians from their predators (Barthalmus, 1994; Trueb, 2003; Wells, 2007). The mucous glands tend to be much more numerous than granular glands, and can secrete mucus to keep the body surface moist and lubricated (Toledo and Jared, 1995; Wells, 2007), and facilitate gaseous diffusion (Trueb, 2003). In addition, the mucous glands are usually more widely distributed than granular glands (Duellman and Trueb, 1994; Toledo and Jared, 1995). Although the precise distribution of the mucous glands differs among species, they usually are distributed over the entire surface of the skin (Le Quang Trong, 1971, 1975a, b). Aside from the granular glands and mucous glands, lipid glands and mixed glands are also discovered in the skin of some amphibians (Blaylock *et al.*, 1976; Seki *et al.*, 1995; Staub and Paladin, 1997; Warburg *et al.*, 2000; Nosi *et al.*, 2002; Tsurada *et al.*, 2002; Lu *et al.*, 2004; Bingol-Ozakpinar and Murathanoglu, 2011). However, only the mucous and granular glands were discovered in *B. pinchonii* and *H. chinensis*. The mucous glands are smaller than the granular glands in both species examined, which is consistent with the results made by Quay (1972), Duellman and Trueb (1994), and Toledo and Jared (1995). Furthermore, the size of the granular and mucous glands of *B. pinchonii* is smaller than those of *H. chinensis* (Table 2), which may be correlated to their different habitats. As an aquatic species, *B. pinchonii* may have fewer predators than the terrestrial species *H. chinensis*, so the granular glands, as a defensive function, of *B. pinchonii* are smaller than those of *H. chinensis*. The secretion of the mucous glands can prevent water loss from body, which is the reason that the mucous glands of *H. chinensis*, as a terrestrial species, are larger than those of *B. pinchonii*, so as to secrete more mucus to keep its body surface moist and lubricated.

Skin thickness varies among amphibian species (Toledo and Jared, 1993). In one species, it is related to habitat (Bani, 1982; Toledo and Jared, 1993), sex (Greven *et al.*, 1995; Schwinger *et al.*, 2001), cellular shape, and cell numbers (Lillywhite *et al.*, 1997), even be enhanced during reproduction (Greven *et al.*, 1995). So, it is difficult to interpret the differences of skin thickness data (Waller and Maibach, 2005). In the present study, the thicknesses of epidermis, stratum spongiosum and stratum compactum, and total thickness of the skin are varied between two species, and have significant difference ($P < 0.001$). We failed to interpret these differences because of absenting fully data, but these may be shown the species-specific differences.

In conclusion, the basic morphological structures of the dorsal skin of *B. pinchonii* and *H. chinensis* are similar, but the distinctions of the distribution of capillary vessels and gland cells, and the thickness of skin are identified. The capillary vessels present in the epidermis of *B. pinchonii*, but absent in *H. chinensis*, can help assist breathing in water. The size of the granular and mucous glands of *H. chinensis* is bigger than that of *B. pinchonii*, which can provide more defenses and prevent water loss on the land. These differences are related to their different habitats, and show a morphological adaptation to environment.

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